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Visualization of defects on a cultured cell layer by utilizing chemical imaging sensor

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Abstract

The chemical imaging sensor is a field-effect sensor which is able to visualize both the distribution of ions (in LAPS mode) and the distribution of impedance (in SPIM mode) in the sample. In this study, a novel wound-healing assay is proposed, in which the chemical imaging sensor operated in SPIM mode is applied to monitor the defect of a cell layer brought into proximity of the sensing surface. A reduced impedance inside the defect, which was artificially formed in a cell layer, was successfully visualized in a photocurrent image.

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Keywords: chemical imaging sensor; SPIM, impedance, wound-healing assay

1. Introduction

In-vitro wound-healing assay [1], which examines the recovery process of a defect in a cultured cell layer, provides important information of migration and growth properties of cells. However, the evaluation method of the recovery process has been mainly limited to optical microscopic observation. For epithelial cells, the so-called transepithelial electrical resistance (TEER) has been used as an index of the tight-junction function in the cell layer. In this study,

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we propose application of the chemical imaging sensor to monitoring of the wound-healing process by visualizing the distribution of impedance in a cell layer.

The chemical imaging sensor [2] is a semiconductor-based chemical sensor, which can visualize the distribution of ions in the LAPS [3] mode and the distribution of impedance in the SPIM [4] mode. In both operation modes, the sensor plate is scanned with a modulated and focused light beam, which generates a photocurrent. In the LAPS mode, the bias voltage is set in the depletion region so that the current is dependent on the Nernst potential. In the SPIM mode, on the other hand, the bias voltage is set in the inversion region, where the current is no more dependent on the Nernst potential, but dependent on the impedance of the sample.

2. Experiment

Figure 1a shows a schematic view of the measurement system. It consists of a sensor plate, which is an insulated n-type silicon, a measurement well mounted on the sensing surface, scanning optics and measurement circuit. A bias voltage is applied between the reference electrode and the sensor plate, and the photocurrent is recorded as a function of the position while scanning the sensor plate with a light beam. In the SPIM mode, the capacitance C_d in the equivalent circuit in Fig.1b is made very small by the negative bias and the current measured in the external circuit is determined by the impedance of the cell layer $Z_{cell\ layer}$.

In this study, the cell layer was not cultured directly on the sensing surface, but was cultured on a permeable membrane at the bottom of a cell culture insert, Millicell® (Merck Millipore), which is often used for TEER measurement. The cell culture insert was stored in an incubator and was put on the sensing surface only when the measurement was carried out. In the first experiment, a resin test pattern formed on the permeable membrane was

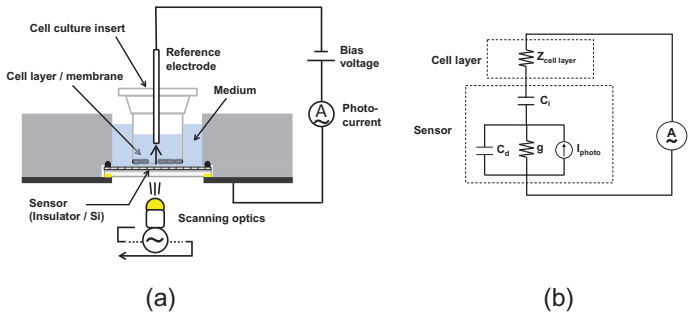

Fig. 1. (a) Schematic view of the experimental setup. (b) Equivalent circuit of the system, where $Z_{cell\ layer}$ is the impedance of the cell layer, C_i and C_d are the capacitance of the insulating layer and that of depletion layer, respectively, and g is the conductance of the depletion layer.

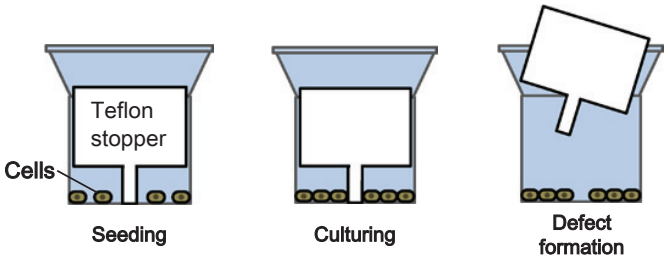

Figure 2: Formation of a defect pattern in a cell layer using a Teflon stopper.

used as a model of a defect in a cell layer. In the second experiment, human colon adenocarcinoma (Caco-2), a typical epithelium cell line, was cultured on the permeable membrane to form a cell monolayer. As shown in Figure 2, a Teflon stopper with a diameter of 1 – 2 mm was placed on the membrane during the culture to form a circular defect with a defined size.

3. Result and discussion

Figure 3 shows a schematic view of the resin pattern formed on the permeable membrane and an example of corresponding photocurrent image obtained by the chemical imaging sensor. A photocurrent pattern corresponding to the ring shape of the resin pattern was clearly observed, which proved that the impedance distribution on the permeable membrane could be visualized by this measurement system. It was also found that the distance between the membrane

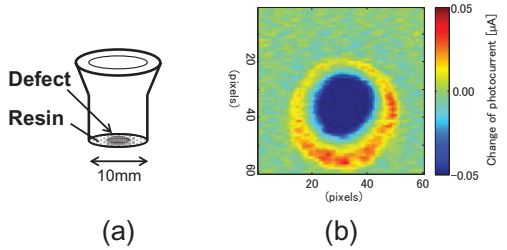

Figure 3: (a) A round-shaped resin pattern on the membrane. (b) Photocurrent image of the resin pattern.

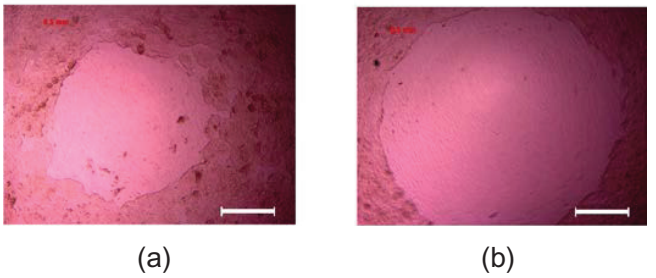

Figure 4: Round-shaped defects (wounds) in Caco-2 cell layers. The Teflon stoppers with diameters of 1 mm and 2 mm were used for (a) and (b), respectively. (scale bar is 0.5 mm)

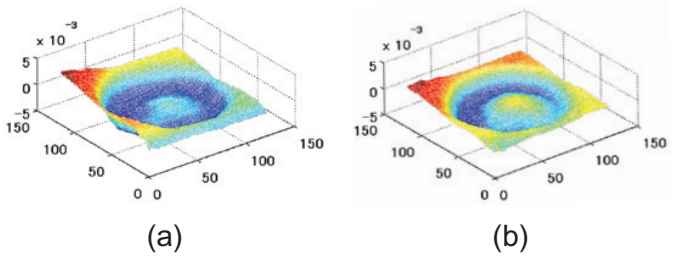

Figure 5: Photocurrent images of defects with diameters of (a) 1 mm of and (b) 2 mm obtained in the SPIM mode.

and the sensing surface was important to obtain better contrast and reproducibility (data not shown). We therefore developed a mechanism to gently press the permeable membrane onto the sensing surface with a small load.

In the second experiment, cultured cell layers with artificial defects were examined. To obtain a uniform cell layer with a clearly defined shape of defect, the cells were seeded on the membrane at low density and were cultured for about 9 days with a Teflon stopper. Figures 4a and 4b show examples of defects formed with Teflon stoppers with diameters of 1 mm and 2 mm, respectively, in which uniform monolayers with clear round-shaped defects were obtained.

Figures 5a and 5b show photocurrent images of defects with diameters of 1 mm and 2 mm, respectively. The peak of the photocurrent was observed at the center of the defect, while the photocurrent was suppressed in the surrounding area. The shape and the size of the photocurrent pattern corresponded to those of defects, showing that visualization of impedance distribution in a cell layer was possible by this method.

4. Conclusion

In this study, the possibility of applying the chemical imaging sensor to the wound-healing assay was studied. A cell monolayer with an artificial defect was prepared on a permeable membrane, which was brought into proximity of the sensing surface, and the photocurrent image was obtained in the SPIM mode using a scanning laser beam. The correspondence between the photocurrent pattern and the defect suggested the possibility of a novel method of wound-healing assay to study the recovery of barrier function of a cell layer.

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